

TRANSACTIONS of the AMERICAN FISHERIES SOCIETY

Volume 117

May 1988

Number 3

Transactions of the American Fisheries Society 117:221-231, 1988
© Copyright by the American Fisheries Society 1988

Factors Regulating Florida Largemouth Bass Stocking Success and Hybridization with Northern Largemouth Bass in Aquilla Lake, Texas

MICHAEL J. MACEINA¹ AND BRIAN R. MURPHY

*Fish Genetics Laboratory, Department of Wildlife and Fisheries Sciences
Texas A&M University, College Station, Texas 77843, USA*

J. JEFFERY ISELY²

*Department of Zoology, North Carolina State University
Raleigh, North Carolina 27695, USA*

Abstract.—We electrophoretically assayed four enzyme-encoding loci in, and determined the ages of, 1,534 largemouth bass *Micropterus salmoides* collected from a new Texas reservoir (Aquilla Lake) over a 41-month period. We used these data to evaluate stocking success and subsequent hybridization of the Florida subspecies *M. s. floridanus* (1.5–6.5 cm total length and age 0 when stocked between 1983 and 1985) with the indigenous northern subspecies *M. s. salmoides*. After these stockings, genomic inflow into the population from Florida largemouth bass was rapid. In the 1986 year class, age-0 Florida largemouth bass, first-generation (F_1) hybrids between the two subspecies, and second- or higher-generation (F_2) hybrids were numerically dominant (72%). For these individuals, the frequencies of Florida alleles at two diagnostic loci (fixed allelic differences between subspecies) were 0.51 and 0.52, respectively. Although the subspecies hybridized extensively in 1986, the population at age 0 did not conform to expected Hardy-Weinberg genotypic proportions because the northern subspecies tended to breed earlier than the Florida subspecies. Higher relative survival rates were evident for hybrid and Florida largemouth bass than for the northern subspecies, and a size-dependent fecundity advantage for Florida largemouth bass females was due to their larger size by age 3.

The largemouth bass *Micropterus salmoides* is a popular sport-fish species widely distributed throughout North America (MacCrimmon and Robbins 1975). Two distinct subspecies, the Flor-

ida largemouth bass *M. s. floridanus* and the northern largemouth bass *M. s. salmoides*, are recognized on the basis of meristic characters (Bailey and Hubbs 1949) and electrophoretically detectable differences in biochemical genetic variation (Philipp et al. 1983). Florida largemouth bass are native to peninsular Florida. Natural hybridized populations of the two subspecies occur in the southeastern United States from northern Florida to Mississippi and northward along the Atlantic coast to Maryland (Philipp et al. 1983). Florida

¹ Present address: South Florida Water Management District, Post Office Box 24680, West Palm Beach, Florida 33416, USA.

² Present address: National Marine Fisheries Service, 3500 Delwood Beach Drive, Panama City, Florida 32407, USA.

largemouth bass have been stocked outside their native range, primarily to enhance sportfishing.

Survival of Florida largemouth bass in northern temperate environments may be lower than that of northern largemouth bass, because the Florida subspecies appears to be less cold-tolerant (Cichra et al. 1982). Philipp et al. (1983) suggested that promiscuous stockings of Florida largemouth bass may introduce maladaptive genes into native northern largemouth bass populations because the two subspecies readily hybridize (Pelzman 1980; Isely et al. 1987).

Previous comparative studies of the growth, survival, and angling catchability of these two subspecies and their hybrids outside Florida have yielded conflicting results (Addison and Spencer 1972; Zolczynski and Davies 1976; Bottroff and Lembeck 1978; Inman et al. 1978; Nieman and Clady 1979; Clady 1980; Smith and Wilson 1980; Wright and Wigtil 1980), perhaps caused by variation in environmental and biological factors among study locations. Inconsistent results may also be due to incorrect subspecific identification. Meristic characters do not provide an unequivocal means to separate various strains (Pelzman 1980) and some Florida hatchery stocks were known to be contaminated with northern alleles (Philipp et al. 1983).

After introductions of Florida largemouth bass into a new Texas reservoir, we examined some of the factors that regulated the gene flow of this subspecies into the indigenous, northern largemouth bass population inhabiting the reservoir. We determined electrophoretically the contribution of stocked Florida largemouth bass to corresponding year classes, and estimated subsequent relative survival, temporal spawning patterns, growth, and fecundity of different largemouth bass strains in the reservoir. Size-dependent mortality was examined during these cohorts' first year of life, and we related such mortality to spawning and early growth of the two subspecies and their hybrids. Finally, we considered the possible long-term effects and management implications this introduction may have on the native population.

Methods

Aquilla Lake (31°55'N, 97°10'W) has a surface area of 1,330 hectares at 163.9 m above mean sea level, contains 6.45×10^7 m³ of water, and has maximum and mean depths of 15.2 and 4.9 m, respectively. Total nitrogen, total phosphorus, and chlorophyll-*a* concentrations indicate the lake is

eutrophic (Maceina and Cichra 1987). Before impoundment began on 29 April 1983, existing excavation pits scheduled for flooding were treated with rotenone and stocked in May 1982 by the Texas Parks and Wildlife Department with age-0 Florida largemouth bass ($N = 31,900$; total length, TL = 2.0–3.0 cm) and bluegills *Lepomis macrochirus*. From 1983 through 1985, three additional May stockings introduced, altogether, 466,450 age-0 Florida largemouth bass (1.5–6.5 cm TL) directly into Aquilla Lake. All fish came from the Texas Parks and Wildlife Department fish hatchery in Huntsville, Texas. These hatchery fish were not assayed electrophoretically before stocking. Subsequent evidence indicated that the 1982 stock was contaminated with northern alleles (43% at discriminant loci), but that the 1983–1985 stocks were Florida largemouth bass (Maceina 1987). Native northern largemouth bass inhabited the watershed upstream from the dam site before impoundment.

Fish collection and laboratory procedures.—Fish ($N = 1,584$) were collected periodically between February 1984 and June 1987 from Aquilla Lake with direct-current electrofishing gear. They were placed on ice after capture, transported to the laboratory, measured (TL, mm), and weighed (0.1 g; fish larger than 1 kg were weighed to the nearest 10 g). Sex was determined by dissection, and otoliths were cleared in glycerine for 1–2 weeks. Liver samples were excised from 1,534 fish and stored at -80°C until electrophoresis was conducted.

Hatch time, age, growth, and fecundity.—Hatch time (from which subspecific temporal spawning patterns were inferred), mean daily growth rates, and ages (d) were estimated from daily ring counts formed on otoliths of age-0 fish (Miller and Storck 1982; Isely and Noble 1987) collected in August and October 1984 ($N = 65$) and in August 1986 ($N = 101$). Average daily growth rate was computed by dividing TL by age. Age-and-growth analyses of 1- and 2-year-old fish were based on examination of whole otoliths (Hoyer et al. 1985). Otoliths estimated to be at least 3 years old were sectioned (Maceina 1988), because whole otoliths become more difficult to read beyond this age (Hoyer et al. 1985). Fish from this study were validated for age by use of marginal increment distances (Maceina 1987). Length- and weight-at-age comparisons were conducted to compare growth rates among various strains.

Fecundity was determined for 43 gravid fish collected between late February and early May 1987. Ovaries were excised, weighed (to 0.1 g),

and stored in Gilson's solution for 2–4 weeks. Numbers of mature ova (>0.75 mm in diameter; Kelly 1962) were estimated volumetrically from counts of samples made with a microscope and an ocular micrometer.

Electrophoretic procedures.—Horizontal starch gel electrophoresis was conducted for four liver enzyme-encoding loci to discriminate between the two largemouth bass subspecies and their hybrids; these loci included isocitrate dehydrogenase (Idh-B, IUBNC 1.1.1.42 [IUBNC 1984]), aspartate aminotransferase (Aat-B, 2.6.1.1), superoxide dismutase (Sod-A, 1.15.1.1), and galactose-1-phosphate uridylyltransferase (Gal-B, 2.7.7.12). Livers of fish collected between February 1984 and April 1985 were homogenized and centrifuged, and supernatants were assayed electrophoretically with tris-citrate (pH 8.0) gel and electrode buffers (Selander et al. 1971). The Gal-B locus, and other loci in fish less than 100 mm TL, could not be assayed consistently with this procedure. Liver samples collected after April 1985 were sonicated, but not centrifuged. All four loci were resolved with *N*-(3-aminopropyl)-morpholine-citrate gel (pH 6.0) and electrode (pH 6.1) buffers (Clayton and Tretiak 1972) for fish as small as 60 mm TL. Enzyme stains generally followed those of Philipp et al. (1982a) and Harris and Hopkinson (1977).

Locus and allele nomenclature for Idh-B, Aat-B, and Sod-A followed that of Philipp et al. (1983). Allele designation of the Gal-B locus, described by Williamson et al. (1986), also followed Philipp et al. (1983). Discrete electromorph mobilities occur between subspecies for the Idh-B (diallelic) and Aat-B (quadallelic) loci. Florida largemouth bass are polymorphic at the Sod-A locus (Sod-A¹, Sod-A²), whereas the northern subspecies is homozygous for the Sod-A² allele (Philipp et al. 1983). Williamson et al. (1986) reported nearly fixed allelic differences between subspecies for Gal-B (diallelic).

Allozyme migration at each locus was compared to known Florida largemouth bass standards maintained at Texas A&M University. Fish were grouped into four electrophoretic phenotypes: (1) northern largemouth bass, (2) Florida largemouth bass, (3) F₁ hybrids (first-generation hybrids between the northern and Florida subspecies), and (4) F_x hybrids (second- or later-generation hybrids between the two subspecies). Fish were considered as F₁ hybrids if they were heterozygous at Idh-B (Idh-B¹B³) and Aat-B (Aat-B¹B³, -B²B³, -B¹B⁴, or -B²B⁴). Most fish (98.6%) hetero-

zygous at these two loci were also heterozygous at Gal-B (Gal-B¹B³). Electrophoretic phenotypes that were not identified as either parental or F₁ were classified as F_x.

Electrophoretic analysis does not always ensure correct identification of these phenotypes. If two loci display fixed differences between these subspecies (Philipp et al. 1983), the error probabilities due to the random recombination of alleles of an F₁ × F₁ cross would be (1/4)², or 12.5%, for production of the Florida or northern phenotype, and (1/2)², or 25%, for production of the F₁ phenotype. The cross of parental subspecies × F₁ would produce the parental and F₁ phenotype (1/2)² × 2, or 50%, of the time, even though the progeny of this cross technically are all F_x hybrids. In interbreeding populations that contain a high proportion of hybrids among the parental subspecies, electrophoretic misidentification is compounded. Because a low percentage (2–14%) of adult (age-2 or older) hybrid largemouth bass contributed to progeny collected in this study (Maceina 1987), phenotype classification errors were probably small and inclusion of the Sod-A locus helped to reduce these errors.

Data analysis.—We pooled data for sample dates within calendar years to determine temporal differences in the proportion of the various phenotypes collected and to examine changes in allele frequencies. By use of length-frequency distributions of age-0 and age-1 fish, we evaluated size-dependent mortality for three year classes (Shelton et al. 1979; Boxrucker 1985). We compared percentages over time with the *z*-statistic (Mendenhall 1971), to assess the relative survival of phenotypes for each year class. Homogeneity of allele frequencies was examined with the *G*-test (Sokal and Rohlf 1981); frequency differences were delineated with a modification of Student's *t*-test (Spiess 1977):

$$(p_1 - p_2) / (p_1 q_1 / 2N_1 + p_2 q_2 / 2N_2)^{1/2};$$

p and *q* = 1 - *p* are frequencies of alternate alleles at a locus, 1 and 2 are different years, and 2*N* (for diploids) is sample size. Deviations from expected Hardy-Weinberg proportions for locus genotypes were tested by chi-square analysis.

Other data were analyzed with the statistical procedures offered by the Statistical Analysis System (SAS 1985). Growth rates and hatching-date distributions of phenotypes were compared by Student's *t*-tests and by one-way analysis of variance and the Student-Newman-Keuls test (Sokal and Rohlf 1981). Correlation coefficients and

TABLE 1.—Percent composition of various largemouth bass phenotypes for four Aquilla Lake year classes over time. Percentages in rows without a letter in common are significantly different ($z > 1.645$, $P < 0.05$, one-tailed test). FLMB = Florida largemouth bass; NLMB = northern largemouth bass; F_1 = first-generation hybrid between FLMB and NLMB; F_x = second- or higher-generation hybrid between FLMB and NLMB. N = sample size.

Year class	Phenotype and sample size	Percent composition in year			
		1984	1985	1986	1987
1983	FLMB	15 z	27 y		
	NLMB	83 z	73 y		
	F_x	2 z	0 z		
	N	133	49		
1984	FLMB	12 z	22 zy	31 yx	42 x
	NLMB	82 z	69 y	57 yx	39 x
	F_1	2 z	8 zy	7 zy	18 y
	F_x	3 z	1 z	4 z	0 z
	N	121	90	94	33
1985	FLMB		12 z	12 z	12 z
	NLMB		64 z	47 y	31 x
	F_1		20 z	35 y	49 x
	F_x		4 z	6 z	8 z
	N		123	345	146
1986	FLMB			33 z	32 z
	NLMB			29 z	27 z
	F_1			32 z	28 z
	F_x			7 z	12 y
	N			203	155

regression equations were computed to describe various relationships.

Results and Discussion

Genetic Composition of Stocked Populations

Florida and hybrid largemouth bass stocked into Aquilla Lake made up only 16% of the 1982 year class in 1984 and 1985 (Maceina 1987). The Florida subspecies made up 15% of the 1983 year class at age 1 and 12% of the 1984 and 1985 year classes at age 0 (Table 1). Despite the stocking of Florida largemouth bass, the indigenous northern subspecies dominated these year classes (Table 1). At age 0, hybrid F_1 and F_x fish made up 5% of the 1984 year class and probably represented offspring from the mating of age-2 Florida and hybrid fish of the 1982 year class with the northern subspecies (Table 1). The 1985 year class contained a higher proportion of F_1 hybrids, which likely resulted from matings of 2-year-old Florida and northern largemouth bass of the 1983 year class.

Most Florida largemouth bass of the 1985 year class were probably stocked fish, not progeny of the 1983 stock. There was a significant difference

($t = 2.71$; $P < 0.01$) in the frequency of the Sod-A¹ allele between the 1983 year class at age 2 (0.15), when these fish were mature, and the 1985 year class at age 0 (0.47). As Williamson et al. (1986) found for another population, we noted nearly fixed subspecific electromorph differences for the Gal-B locus ($N = 245$); only one questionable heterozygote was observed, an individual that other loci indicated was a Florida phenotype. All northern largemouth bass, identified by the Idh-B¹ and Aat-B¹ and -B² alleles, were homozygous for the Gal-B¹ allele.

Factors Regulating Introgression of Florida Alleles

Because densities of fish were not determined, actual survival rates of phenotypes could not be computed. However, relative abundances of phenotypes were compared over time as an indicator of relative survival in the lake. No attempt was made to distinguish between fishing and natural mortality. Because boat access to the lake was limited as the reservoir filled before 1985, and because a minimum legal length of 406 mm TL was imposed 1 October 1985, fishing effects on phenotype distributions at that time were probably small (Maceina 1987).

Florida and hybrid largemouth bass of the 1983–1985 year classes increased their relative abundances during the study period, indicating that their survival was probably higher than that of northern largemouth bass (Table 1). The percentage of Florida largemouth bass captured from the 1985 year class remained stable (12%) through time, but F_1 hybrid abundance increased 2.5-fold while the percentage of northern largemouth bass declined by half. In 1985, age-0 northern largemouth bass were the dominant (64%) phenotype collected, but, in 1987 at age-2, these fish made up only 31% of this year class. In the 1984 and 1985 year classes, the relative abundances of F_x fish remained about the same (0–8%) over time, but increased between 1986 and 1987 for the 1986 year class.

In addition to phenotypic differences in survival, size-dependent mortality also may have occurred during the first winter for young largemouth bass from the 1984 year class; the percentage of small fish (<150 mm TL) in the bimodal distribution declined ($z = 2.09$; $P < 0.05$) from 44 to 27% (Figure 1A). This percentage change suggested that higher survival rates occurred for larger fish than for smaller fish. In support of this, the condition of these fish declined

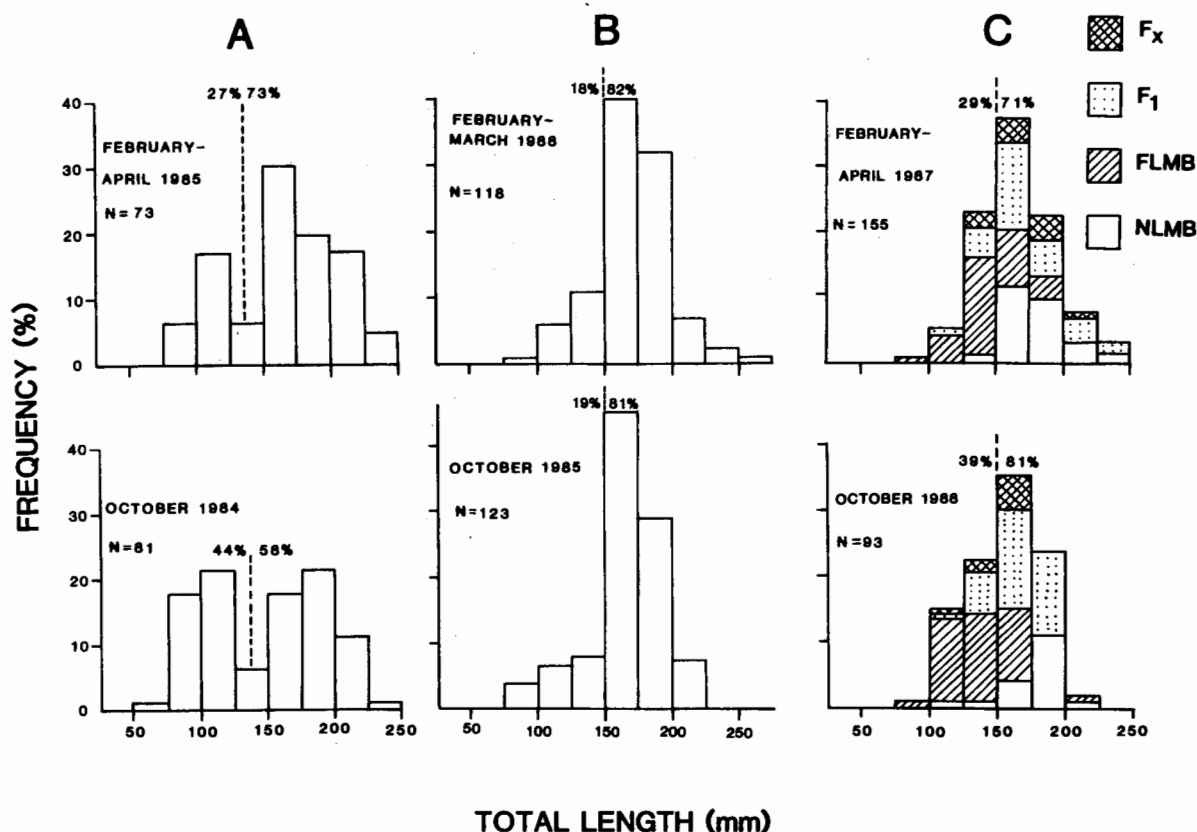


FIGURE 1.—Length-frequency distributions for the 1984 (A), 1985 (B), and 1986 (C) year classes of largemouth bass collected from Aquilla Lake, Texas, at age 0 (bottom) and age 1 (top). All phenotypes were combined for the 1984 and 1985 year classes. FLMB = Florida largemouth bass; NLMB = northern largemouth bass; F_1 = first-generation hybrid between FLMB and NLMB; F_x = second- or higher-generation hybrid between FLMB and NLMB. Percentages either side of vertical broken lines are aggregate frequencies for small (<150 mm) and large fish.

during this time (Maceina and Murphy 1988), although growth of both Florida and northern largemouth bass increased (Table 2). However, evidence of size-dependent winter mortality of young fish from the 1985 year class was not apparent (Figure 1B). Growth was negligible between October 1985 and February–March 1986 (Table 2) and condition declined for these fish (Maceina and Murphy 1988), but the relative proportions of small and large fish remained the same. Higher winter mortality may have occurred in the 1984 year class because average ambient air temperatures were lower between December 1984 and February 1985 than for corresponding months 1 year later (U.S. National Climatic Center 1984–1986).

Positive correlations ($r = 0.62$ to 0.80 ; $P < 0.01$) were computed between age (d) and length at capture for various age-0 largemouth bass phenotypes in the 1984 and 1986 year classes. Fish that hatched earlier tended to be the larger fish later in the growing season. Further, there also was a positive re-

lationship between age and average daily growth rate. These two reinforcing trends resulted in a wide range of northern largemouth bass lengths in October 1984 (Maceina and Isely 1986). The smaller of these fish were in poorer condition, and few prey were small enough for them to eat (Maceina and Isely 1986). Thus, late-hatched, resident northern largemouth bass in this year class were less likely to survive their first winter than early-hatched fish. Size-dependent mortality during the first year could be an important factor affecting phenotype proportions because the two subspecies displayed different temporal spawning patterns.

Based on evidence from surviving fish, northern largemouth bass tended to spawn earlier than the Florida subspecies in 1986, a year when fish were not stocked into the lake (Table 3). Spawning of northern largemouth bass was estimated to have begun on 5 March, but intraspecific mating between the two subspecies and mating among individual Florida largemouth bass began 16 d and 23 d later, respectively. Conversely, the youngest

TABLE 2.—Mean total lengths (TL in mm), weights (WT in g), and number (N) of various largemouth bass phenotypes collected from the 1983–1986 year classes in Aquilla Lake. Values for mean lengths or weights in rows for a particular year class and sample time followed by the same letter are not significantly different ($P > 0.05$). FLMB = Florida largemouth bass; NLMB = northern largemouth bass; F_1 = first-generation hybrid between FLMB and NLMB; F_x = second- or higher-generation hybrid between FLMB and NLMB. Asterisks denote N less than 5.

Sample time	Fac-tor	Year class										
		1983		1984		1985			1986			
		FLMB	NLMB	FLMB	NLMB	FLMB	NLMB	F ₁	FLMB	NLMB	F ₁	F _x
Feb-Apr 1984	TL	167 z	171 z									
	WT	61 z	71 z									
	N	9	52									
Aug-Oct 1984	TL		286	139 z	157 z							
	WT		358	40 z	63 z							
	N	*	31	11	58							
Dec-Apr 1984- 1985	TL	295 z	306 z	168 z	173 z							
	WT	365 z	415 z	61 z	76 z							
	N	22	63	16	100							
Oct 1985	TL					157 z	168 z	165 z				
	WT					47 z	59 z	58 z				
	N					15	79	24				
Feb-Mar 1986	TL		407	327 z	322 z	177 z	177 z	163 z				
	WT		1,253	508 z	500 z	54 z	62 z	49 z				
	N	*	5	19	36	5	64	41				
Aug 1986	TL					213 z	240 y	235 y	91 z	123 y	120 y	104 z
	WT					139 z	211 y	196 y	11 z	28 y	24 y	16 z
	N					28	68	52	30	43	30	6
Oct 1986	TL			370 z	354 z	272 z	286 z	280 z	138 z	180 x	164 y	149 z
	WT			756 z	673 z	285 z	328 z	313 z	32 z	77 x	55 y	42 z
	N			10	18	9	29	29	36	15	34	8
Feb 1987	TL					253 z	285 y	295 y	142 z	177 y	175 y	161 y
	WT					209 z	314 y	355 y	31 z	65 y	64 y	49 y
	N					13	28	31	22	28	16	8
Apr 1987	TL					275 z	310 z	302 z	146 z	187 y	176 y	186 y
	WT					265 z	422 z	383 z	37 z	83 y	70 y	83 y
	N					5	17	40	28	14	28	11
Feb-May ^a 1987	TL			427 z	377 y							
	WT			1,478 z	808 y							
	N			14	13							

^a Collections were combined to provide sufficient sample sizes.

Florida subspecies hatched 9 d later than the youngest northern largemouth bass in the cohort, suggesting that northern largemouth bass ended spawning before the Florida subspecies. The F_1 hybrids had a smaller range of ages than the parental subspecies, and a smaller age variance than the northern subspecies (Table 3), also demonstrating a partially segregated spawning period for each subspecies. Some individual Florida largemouth bass began spawning with the northern subspecies early in their mating season because the average age of young F_1 hybrids was older than that of either set of purebred offspring (Table 3). Isely et al. (1987) suggested that this may be due to subspecies differences in maturation between the sexes. Nevertheless, subspecies spawning time

TABLE 3.—Summary statistics on age distributions from various young-of-the-year largemouth bass phenotypes collected from Aquilla Lake on 5 August 1986. Mean ages and variances followed by the same letter are not significantly different ($P > 0.05$). FLMB = Florida largemouth bass; NLMB = northern largemouth bass; F_1 = first-generation hybrid between NLMB and FLMB. N = sample size.

Age statistics (d)	Phenotype		
	FLMB ($N=29$)	F_1 ($N=30$)	NLMB ($N=42$)
Mean	105 z	125 y	117 x
Variance	226 zy	139 z	301 y
Minimum	69	85	78
Maximum	130	137	153
Mode (10-d interval)	94–103	124–133	114–123

overlapped and F_1 progeny made up 32% of the 1986 year class at age 0 (Table 1).

Parker et al. (1985) reported no differences in development rates from fertilization to the free-swimming stage between progeny produced by Florida parents and a northern \times Florida cross, indicating that early development rate probably was not a variable in our analysis for these two phenotypes. In addition, we assumed that hatch dates were indicative of spawning time and that differential mortality before sampling was negligible. Thus, the period of nonrandom mating (i.e., mating only within the subspecies) contributed in part to the high percentage (33%) of Florida largemouth bass among young of the year in 1986, a year in which no stocking occurred (Table 1).

Analysis of Hardy-Weinberg equilibria corroborated our findings that the subspecies did not mate randomly in 1986. Chi-square values in tests for disequilibrium at the Idh-B, Aat-B, and Gal-B loci for these fish at age 0 ranged from 17.81 to 21.95 ($P < 0.01$). Disequilibrium was caused by a deficiency of northern-Florida heterozygotes and a surplus of subspecific homozygous genotype combinations at these loci. Spatial and behavioral differences (e.g., subspecies recognition during breeding) may have inhibited random mating.

For fish of the 1986 year class collected in August 1986, there was no relationship between average daily growth rate and hatching time (Maceina 1987). However, average daily growth rate differed significantly ($P < 0.05$) among phenotypes and was highest for northern fish (1.05 mm/d), lowest for Florida fish (0.87 mm/d), and intermediate for F_1 hybrids (0.96 mm/d). As a result of these differences and subspecific variation in hatch date, northern largemouth bass were larger than Florida largemouth bass in August 1986 (Table 2). Although average daily growth rate was less for F_1 hybrids than for the northern subspecies, these fish were similar in length to northern largemouth bass in August 1986 (Table 2), because F_1 hybrids were older, on average, than the northern subspecies (Table 3). Subspecific variation in daily growth rates apparently continued through October 1986, the size of F_1 hybrids remaining intermediate between those of the subspecies (Table 2). The F_x hybrids were smaller than northern and F_1 largemouth bass, but similar in size to the Florida subspecies (Table 2). However, F_1 and F_x growth continued after October during the winter months, while northern and Florida largemouth bass growth did not. By April 1987, size differences were not apparent among

northern, F_1 , and F_x fish, but the Florida fish remained smaller (Table 2; Figure 1C). Between 1986 and 1987, there were no reductions in percentages of Florida and hybrid phenotypes in the population (Table 1), and size-dependent mortality had not occurred (Figure 1C). Therefore, higher hybrid growth rates were not a reflection of lower survival rates of smaller individuals in the cohort.

For fish of the 1983 and 1984 year classes, growth differences were not apparent between subspecies at age 2 and younger (Table 3). For these fish, sex-related differences in growth rates were also not evident. By age 3, however, Florida largemouth bass from the 1984 year class exceeded northern largemouth bass in size, and thus recruited to the fishery (406 mm TL minimum) at an earlier age. This was due to the rapid growth between age 2 and age 3 by the female Florida fish; females collected in spring 1987 averaged 446 mm TL and 1.72 kg, significantly higher values ($t = 2.20$ and 2.23 , respectively; $P < 0.05$) than those of female northern largemouth bass (391 mm TL and 0.93 kg). Thus, the higher growth rates observed for the Florida subspecies appeared to be genetically and sexually related. However, comparison of age-1 and age-2 sizes among the 1985 year class collected in fall 1986 and spring 1987 indicated that growth was less for Florida than for northern and F_1 hybrid largemouth bass for two of four comparisons (Table 2). Whether or not the high proportion of F_1 hybrids present in this year class resulted in lower growth of Florida largemouth bass due to intraspecific competition remains unknown.

Faster growth rates observed for female Florida largemouth bass collected from the 1984 year class in spring 1987 conferred a size-dependent fecundity advantage to these fish compared to their northern counterparts (Table 4). There were no significant differences in number of mature ova per gram of ovary among phenotypes (Table 4). Hence, for all phenotypes (range, 262–531 mm TL), a pooled quadratic equation predicting fecundity (FEC) from length (TL in mm) was computed ($N = 43$; $P < 0.01$; $r^2 = 0.71$):

$$FEC = 51,770 - 340.6(TL) + 0.648(TL^2).$$

Thus, production of mature ova increases quadratically as fish grow in length. This suggests that greater production of Florida and hybrid largemouth bass progeny could be expected from the Florida than from the northern female. Predicted fecundity estimates from this regression were similar to direct counts made for phenotypes in each

TABLE 4.—Mean number of mature ova per gram of ovary and fecundity (number of ova >0.75 mm diameter) for largemouth bass phenotypes from the 1984 and 1985 year classes in Aquilla Lake collected in spring 1987. Corresponding predicted fecundity estimates from the regression equation^a are presented (TL = total length in mm). For a particular year class, mean values in rows followed by the same letter are not significantly different ($P > 0.05$). FLMB = Florida largemouth bass; NLMB = northern largemouth bass; F₁ = first-generation hybrid between FLMB and NLMB). *N* = sample size.

Factor	Year class				
	1984		1985		
	FLMB	NLMB	FLMB	NLMB	F ₁
<i>N</i>	7	5	2	12	17
Number of ova per gram of ovary	543 z	434 z	476 z	533 z	554 z
Fecundity	32,500 z	12,600 y	7,100 z	9,200 z	10,200 z
Predicted fecundity ^a	28,800	17,700	7,000	8,200	8,100

^a Fecundity = $51,700 - 340.6(TL) + 0.648(TL^2)$.

year class (Table 4). If F₁ hybrids display differential fertility compared to either subspecies, it was not evident with respect to the production of mature ova in these fish.

Assessment

The Florida subspecies displayed certain advantages as well as disadvantages related to the maintenance of Florida alleles in the Aquilla Lake population. Besides the greater fecundity it brings, the subspecies' faster growth rate by age 3 can provide a foraging advantage because largemouth bass select the largest prey they can (Shelton et al. 1979; Schramm and Maceina 1986; Maceina 1987). This faster growth rate, however, caused female Florida largemouth bass became recruited to the Aquilla Lake fishery at an earlier age than was the northern subspecies. Because Florida fish spawn later than the northern subspecies and because their progeny grow more slowly during their first year (Isely et al. 1987; this study), these smaller juvenile fish could be at a disadvantage if their food became limited (Shelton et al. 1979; Maceina and Isely 1986) or if they were exposed to adverse weather conditions (Summerfelt 1975). Thus, size-dependent mortality would be more likely for these first-year fish. The interacting variables of spawning time, growth, and survival undoubtedly will affect allele frequency distributions and phenotype proportions in the Aquilla Lake largemouth bass population over time.

At the Idh-B, Aat-B, and Gal-B loci, Florida alleles increased substantially over time in the 1982–1985 year classes (Table 5). The frequency of Florida alleles at these loci reached 50% in the 1984 year class at age 3 and in the 1986 year class at age 1. The 1986 year class (which was not sup-

plemented by stocking) expressed allele frequencies at Idh-B and Aat-B (Maceina 1987) similar to those predicted from the regression equations of Philipp et al. (1985) based on the latitudinal distribution of these alleles in the USA. We hypothesize, however, that the frequency of Florida alleles will continue to increase over time in the population. The high proportion of Florida and hybrid largemouth bass in the 1986 year class (72%), a lower percentage (31–39%) of northern largemouth bass in the 1984 and 1985 year classes by 1987, the size-dependent fecundity advantage for age-3 Florida fish, and the higher survival rates for the Florida and hybrid phenotypes will contribute to the dominance of Florida alleles in successive year classes. Thus, if stable equilibria of alleles are achieved at these diagnostic loci in the future, the frequency of the Florida alleles could be considerably higher than 0.5, although less than 1.0 because northern alleles will persist in the population.

Similarly, Bottroff and Lembeck (1978) found that stocking of Florida largemouth bass (average scale count, 70.4) in two California reservoirs was followed by an increase in mean lateral line scale counts of captured reservoir fish. Counts increased from 63 (average for northern largemouth bass) to 71 over a 16-year period, presumably as a result of dominance by the Florida subspecies and of extensive hybridization. Aquilla Lake may also develop a population predominantly of Florida and hybrid largemouth bass. This would result in a high proportion of introgression, but many of these fish would be classified as Florida largemouth bass because only two discriminate loci currently are known to distinguish each subspecies.

Initially, year-class composition of age-0 Flor-

TABLE 5.—Frequency of Florida largemouth bass alleles in the Aquilla Lake largemouth bass population at the Idh-B, Aat-B, Gal-B, and Sod-A loci over time. For a particular year class, frequencies in rows followed by the same letter are not significantly different ($P > 0.05$). N is the number of alleles. Asterisks denote $P < 0.05^*$ or $P < 0.01^{**}$.

Year class	Allele(s) and sample size	G-test	Allele frequency in year			
			1984	1985	1986	1987
1982	Idh-B ³	7.48**	0.04 z	0.23 y		
	Aat-B ³ +B ⁴	4.66**	0.04 z	0.17 y		
	Sod-A ¹	2.08	0 z	0.03 z		
	N		54	30		
1983	Idh-B ³	6.02*	0.15 z	0.27 y		
	Aat-B ³ +B ⁴	5.12*	0.16 z	0.27 y		
	Sod-A ¹	0.24	0.03 z	0.04 z		
	N		266	98		
1984	Idh-B ³	45.74**	0.15 z	0.26 y	0.36 x	0.52 w
	Aat-B ³ +B ⁴	45.62**	0.15 z	0.27 y	0.36 x	0.52 w
	Gal-B ²	5.06*			0.36 z	0.52 y
	Sod-A ¹	2.14	0.05 z	0.08 z	0.08 z	0.09 z
	N		242	180	188	66
1985	Idh-B ³	18.74**		0.24 z	0.33 y	0.41 x
	Aat-B ³ +B ⁴	13.74**		0.25 z	0.32 y	0.40 x
	Gal-B ²	17.72**		0.23 z	0.33 y	0.40 x
	Sod-A ¹	0.30		0.09 z	0.10 z	0.10 z
	N			246	690	292
1986	Idh-B ³	0.07			0.52 z	0.53 z
	Aat-B ³ +B ⁴	0.38			0.51 z	0.54 z
	Gal-B ²	0.04			0.52 z	0.53 z
	Sod-A ¹	0.08			0.12 z	0.11 z
	N				406	310

ida largemouth bass was moderately low in Aquilla Lake after stocking. Subsequent higher survival rates, hybridization with native northern largemouth bass, and partial subspecific spawning segregation established a population that displayed equal genetic composition of both subspecies by the 1986 year class (2–4 years after stocking). Ambient air temperatures were lower at Aquilla Lake in the winter than at 13 lakes where Florida largemouth bass resided (Philipp et al. 1982b; Maceina and Murphy 1988), and corresponding differences in water temperature likely exist between these regions. Water temperatures in Aquilla Lake ranged from 4 to 31°C during the study, and the reservoir was ice covered for 2 weeks in 1983–1984 (Maceina 1987). Although winter climatic conditions in Aquilla Lake were colder than those typically measured in peninsular Florida, there was no decline in survival of Florida and hybrid largemouth bass relative to that of northern largemouth bass. In addition, these conditions did not adversely affect the condition of Florida and F_1 largemouth bass in Aquilla Lake (Maceina and Murphy 1988). Poorer performance (i.e., lower relative survival after age 0 and slower growth after age 2) exhibited by the indigenous northern

largemouth bass may have been due in part to the population's history. Before impoundment, northern largemouth bass were adapted to environmentally unstable lotic conditions that prevailed in the intermittent Aquilla and Hackberry creeks. Florida largemouth bass, in contrast, primarily evolved in natural lake systems and may have been better adapted to the new lentic conditions of Aquilla Lake.

In conclusion, the introduction of the Florida subspecies into Aquilla Lake appears to be a successful management initiative. However, indiscriminate stocking of Florida largemouth bass could affect northern largemouth bass populations negatively. Aquilla Lake is located only 1°18'N of the most northern pure Florida largemouth bass population described by Philipp et al. (1983). In more northern latitudes, prolonged exposure to cold temperatures may reduce growth, condition, and survival of the Florida subspecies, because it is less tolerant of cold shock than northern largemouth bass (Cichra et al. 1982). It would be useful to have information about the annual thermal regime of a prospective stocking site before one introduces Florida largemouth bass there. In addition, reservoirs or lakes with conditions that can

inflict size-dependent mortality on young largemouth bass (e.g., spring-summer water level declines; prey limitations; excess predators; prolonged cold temperatures) may not be suitable for Florida largemouth bass introductions.

Acknowledgments

Primary financial support was provided by the Fort Worth District of the U.S. Army Corps of Engineers, contract DACW-79-C-0410, as part of Texas Agricultural Experiment Station projects 6536-S and 6843-H, and this paper represents contribution TA-22945. Electrophoretic facilities and supplies were kindly provided by I. F. Greenbaum and were funded by National Institutes of Health grant GM-27014 and National Science Foundation grant DEB-8117447. B. Putman and K. Kulzer conducted some of the electrophoretic analysis. K. Sellers, Texas Parks and Wildlife Department, provided the stocking schedule. Numerous people assisted in collecting and processing fish, and their help is greatly appreciated. J. R. Gold, J. W. Bickham, S. W. Kelsch, C. Cichra, and D. E. Campton provided suggestions to improve the manuscript, and D. Murphy helped in its preparation.

References

- Addison, J. H., and S. L. Spencer. 1972. Preliminary evaluation of three strains of largemouth bass (*Micropterus salmoides*) stocked in ponds in south Alabama. Proceedings of the Annual Conference Southeastern Association of Fish and Wildlife Agencies 25:366-374.
- Bailey, R. M., and C. L. Hubbs. 1949. The black basses (*Micropterus*) of Florida with a description of a new species. University of Michigan Museum of Zoology Occasional Papers 516.
- Bottroff, L. J., and M. E. Lembeck. 1978. Fishery trends in reservoirs of San Diego County, California, following the introduction of Florida largemouth bass, *Micropterus salmoides floridanus*. California Fish and Game 64:4-23.
- Boxrucker, J. 1985. First year growth and survival of stocked largemouth bass in a small Oklahoma impoundment. Proceedings of the Annual Conference Southeastern Association of Fish and Wildlife Agencies 36:369-376.
- Cichra, C. E., W. H. Neil, and R. L. Noble. 1982. Differential resistance of northern and Florida largemouth bass to cold shock. Proceedings of the Annual Conference Southeastern Association of Fish and Wildlife Agencies 34:19-24.
- Clady, M. D. 1980. Results of stocking young largemouth bass and spotted bass in several ratios in Oklahoma ponds. Proceedings of the Oklahoma Academy of Science 60:18-25.
- Clayton, J. W., and D. N. Tretiak. 1972. Amine-citrate buffers for pH control in starch gel electrophoresis. Journal of the Fisheries Research Board of Canada 29:1169-1172.
- Harris, H., and D. A. Hopkinson. 1977. Handbook of enzyme electrophoresis in human genetics. American Elsevier, New York.
- Hoyer, M. V., J. V. Shireman, and M. J. Maceina. 1985. Use of otoliths to determine age and growth of largemouth bass in Florida. Transactions of the American Fisheries Society 114:307-309.
- Inman, C. R., R. C. Dewey, and P. P. Durocher. 1978. Growth comparisons and catchability of three largemouth bass strains. Proceedings of the Annual Conference Southeastern Association of Fish and Wildlife Agencies 30:40-47.
- Isely, J. J., and R. L. Noble. 1987. Validation of daily ring deposition in otoliths of wild young-of-the-year largemouth bass. Texas Journal of Science 39:273-277.
- Isely, J. J., R. L. Noble, J. B. Koppelman, and D. P. Philipp. 1987. Spawning period and first-year growth of northern, Florida, and intergrade stocks of largemouth bass. Transactions of the American Fisheries Society 116:757-762.
- IUBNC (International Union of Biochemistry, Nomenclature Committee). 1984. Enzyme nomenclature 1984. Academic Press, Orlando, Florida.
- Kelly, J. W. 1962. Sexual maturity and fecundity of the largemouth bass *Micropterus salmoides* (Lacepede) in Maine. Transactions of the American Fisheries Society 91:23-28.
- MacCrimmon, H. R., and W. H. Robbins. 1975. Distribution of black basses in North America. Pages 56-66 in H. Clepper, editor. Black bass biology and management. Sport Fishing Institute, Washington, D.C.
- Maceina, M. J. 1987. Evaluation of Florida largemouth bass introductions into a new Texas reservoir: factors regulating stocking success and introgression. Doctoral dissertation. Texas A&M University, College Station.
- Maceina, M. J. 1988. A simple grinding procedure to section otoliths. North American Journal of Fisheries Management 8:141-143.
- Maceina, M. J., and M. F. Cichra. 1987. Limnological characteristics of Aquilla Lake, Texas, during impoundment. Texas Journal of Science 39:55-69.
- Maceina, M. J., and J. J. Isely. 1986. Factors affecting growth of an initial largemouth bass year class in a new Texas reservoir. Journal of Freshwater Ecology 3:485-492.
- Maceina, M. J., and B. R. Murphy. 1988. Variation in the weight-to-length relationship among Florida and northern largemouth bass and their intraspecific F₁ hybrid. Transactions of the American Fisheries Society 117:232-237.
- Mendenhall, W. 1971. Introduction to probability and statistics. Duxbury Press, Belmont, California.
- Miller, S. J., and T. Storck. 1982. Daily growth rings in otoliths of young-of-the-year largemouth bass. Transactions of the American Fisheries Society 111:527-530.

- Nieman, D. A., and M. D. Clady. 1979. Florida and northern largemouth bass: growth and survival in a heated reservoir. *Proceedings of the Oklahoma Academy of Science* 59:47-50.
- Parker, H. R., D. P. Philipp, and G. S. Whitt. 1985. Gene regulatory divergence among species estimated by altered developmental patterns in interspecific hybrids. *Molecular Biology and Evolution* 2: 217-250.
- Pelzman, R. J. 1980. Impact of Florida largemouth bass, *Micropterus salmoides floridanus*, introductions at selected northern California water with a discussion of the use of meristics for detecting introgression and for classifying individual fish of intergraded populations. *California Fish and Game* 66:133-162.
- Philipp, D. P., W. F. Childers, and G. S. Whitt, editors. 1982a. Electrophoretic analyses of largemouth bass, *Micropterus salmoides*. Pages 1-156 in D. P. Philipp, W. F. Childers, and G. S. Whitt, editors. *Biochemical genetics of largemouth bass, Micropterus salmoides*. Electric Power Research Institute, Palo Alto, California.
- Philipp, D. P., W. F. Childers, and G. S. Whitt, editors. 1982b. Correlation of allele frequencies of largemouth bass populations with physical/environmental parameters. Pages 157-209 in D. P. Philipp, W. F. Childers, and G. S. Whitt, editors. *Biochemical genetics of largemouth bass, Micropterus salmoides*. Electric Power Research Institute, Palo Alto, California.
- Philipp, D. P., W. F. Childers, and G. S. Whitt. 1983. A biochemical genetic evaluation of the northern and Florida largemouth bass. *Transactions of the American Fisheries Society* 112:1-20.
- Philipp, D. P., W. F. Childers, and G. S. Whitt. 1985. Correlation of allele frequencies with physical and environmental variables for populations of largemouth bass, *Micropterus salmoides* (Lacepede). *Journal of Fish Biology* 27:347-365.
- SAS (Statistical Analysis System). 1985. SAS user's guide: version 5. SAS Institute, Cary, North Carolina.
- Schramm, H. L., and M. J. Maceina. 1986. Distribution and diet of Suwannee bass and largemouth bass in the lower Santa Fe River, Florida. *Environmental Biology of Fishes* 15:221-228.
- Selander, R. K., M. H. Smith, S. Y. Yang, W. F. Johnson, and J. B. Gentry. 1971. Biochemical polymorphism and systematics in the genus *Peromyscus*. I. Variation in the old field mouse (*Peromyscus polionotus*). Pages 49-90 in *Studies in genetics*, volume 6. University of Texas Publication 7103, Austin.
- Shelton, W. L., W. D. Davies, T. A. King, and T. J. Timmons. 1979. Variation in the growth of the initial year class of largemouth bass in West Point Reservoir, Alabama and Georgia. *Transactions of the American Fisheries Society* 108:142-149.
- Smith, R. P., and J. L. Wilson. 1980. Growth comparisons of two subspecies of largemouth bass in Tennessee ponds. *Proceedings of the Annual Conference Southeastern Association of Fish and Wildlife Agencies* 34:25-30.
- Sokal, R. R., and F. J. Rohlf. 1981. *Biometry*. Freeman, San Francisco.
- Spiess, E. B. 1977. *Genes in populations*. Wiley, New York.
- Summerfelt, R. C. 1975. Relationship between weather and year-class strength in largemouth bass. Pages 166-174 in H. Clepper, editor. *Black bass biology and management*. Sport Fishing Institute, Washington, D.C.
- U.S. National Climatic Center. 1984-1986. Climatic data for Texas, volumes 89-91. Asheville, North Carolina.
- Williamson, J. H., G. J. Carmichael, M. E. Schmit, and D. C. Morizot. 1986. Additional biochemical markers for largemouth bass. *Transactions of the American Fisheries Society* 115:460-465.
- Wright, G. L., and G. W. Wigtil. 1980. Comparison of growth, survival, and catchability of Florida, northern, and hybrid bass in a new Oklahoma reservoir. *Proceedings of the Annual Conference Southeastern Association of Fish and Wildlife Agencies* 34:31-38.
- Zolczynski, S. J., Jr., and W. D. Davies. 1976. Growth characteristics of northern and Florida subspecies of largemouth bass and their hybrid and a comparison of catchability between the subspecies. *Transactions of the American Fisheries Society* 105:240-243.

Received August 23, 1987

Accepted May 25, 1988